

DOCUMENT-IDENTIFIER: US 6727073 B1

TITLE: Method for detecting enteric disease

Brief Summary Text (20):

Diarrhea may be caused by factors other than invasive enteric pathogens, and it is particularly useful for the treating physician to know whether, in the absence of various pathogens that are typically assayed, the diarrhea is associated with an inflammatory condition of the intestines or has other, non-inflammatory--and hence, generally, non-pathogenic--causes. Lactoferrin is an iron-binding bactericidal protein contained in granules in polymorphonuclear (PMN) leukocytes and is found in intestinal secretions, as well as in other secretions. Since the PMNs increase rapidly in number in response to an infection, the number of lactoferrin granules also increases. Studies have shown that the presence of fecal lactoferrin is a reliable general indicator of inflammation in the intestinal tract. See Choi et al.; To Culture or Not to Culture: Fecal Lactoferrin Screening for Inflammatory Bacterial Diarrhea, in: Journal of Clinical Microbiology, April 1996, p. 928-932. Because lactoferrin is also found in breast milk, fecal lactoferrin found in breast-fed infants is not an effective indicator of an inflammatory intestinal condition, as the presence of lactoferrin from breast milk will lead to false positives. Nevertheless, a method for detecting multiple enteric foodborne pathogens that includes a general indicator for an inflammatory condition of the intestines will provide the physician who is treating a patient other than a breast-fed infant with valuable information.

Other Reference Publication (14):

Sugi et al., "Fecal Lactoferrin as a Marker for Disease Activity in Inflammatory Bowel Disease: Comparison with Other Neutrophil-derived Proteins", The American J. of Gastroenterology, vol. 91, pp. 927-934, 1996, USA.

Other Reference Publication (17):

Fine, et al., "Utility of a Rapid Fecal Latex Agglutination Test Detecting the Neutrophil Protein, Lactoferrin, for Diagnosing Inflammatory Causes of Chronic Diarrhea", American J. of Gastroenterology, vol. 93, No. 8, 1998, pp. 1300-1305. USA.

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DOCUMENT-IDENTIFIER: US 20020168698 A1

TITLE: Method for differentiating irritable bowel syndrome from inflammatory bowel disease (IBD) and for monitoring persons with IBD using total endogenous lactoferrin as a marker

CLAIMS:

1. A method for distinguishing irritable bowel syndrome from inflammatory bowel disease, the method comprising: obtaining a fecal sample from a person to be diagnosed; and determining whether said sample contains an elevated level of endogenous lactoferrin, wherein if said sample does contain an elevated level of endogenous lactoferrin, diagnoses of irritable bowel syndrome and other noninflammatory etiologies are substantially precluded.
2. The method as recited in claim 1, further comprising diluting said fecal sample.
3. The method as recited in claim 2, wherein said step of diluting said fecal sample comprises diluting said sample to a 1:400 dilution factor.
4. The method as recited in claim 1, wherein said endogenous lactoferrin comprises total lactoferrin from one or more of plasma, bile, leukocytes and mucosal secretions.
5. The method as recited in claim 1, wherein said endogenous lactoferrin is qualitatively determined.
6. The method as recited in claim 1, wherein said step of determining whether said sample contains an elevated level of endogenous lactoferrin includes contacting said sample with immobilized polyclonal antibodies to human lactoferrin to create a treated sample.
7. The method as recited in claim 6, wherein said step of determining whether said sample contains an elevated level of endogenous lactoferrin further includes contacting said treated sample with enzyme-linked polyclonal antibodies to create a readable sample.
8. The method as recited in claim 7, wherein said step of determining whether said sample contains an elevated level of endogenous lactoferrin further includes determining an optical density of said readable sample at 450 nm, wherein said optical density corresponds to a level of endogenous lactoferrin in the sample.
9. The method as recited in claim 8, wherein if said optical density of said readable sample is greater than 0.200, said fecal sample contains an elevated level of endogenous lactoferrin.
10. An assay for determining the concentration of endogenous lactoferrin, said assay comprising: obtaining a human fecal sample; diluting said fecal sample; contacting said sample with immobilized polyclonal antibodies to endogenous lactoferrin to create a treated sample; contacting said treated sample with enzyme-linked polyclonal antibodies to create a readable sample; determining the optical

density of said readable sample at 450 nm; generating a purified lactoferrin standard curve; and comparing said optical density of said readable sample to said standard curve to determine the concentration of endogenous lactoferrin in said fecal sample.

11. The assay as recited in claim 10, wherein said step of diluting said fecal sample comprises diluting said sample by serial ten-fold dilutions.

12. A diagnostic assay for differentiating irritable bowel syndrome from inflammatory bowel disease by determining the level of endogenous lactoferrin, said assay comprising: obtaining a human fecal sample; diluting said sample; contacting said sample with immobilized polyclonal antibodies to endogenous lactoferrin to create a treated sample; contacting said treated sample with enzyme-linked polyclonal antibodies to create a readable sample; and determining the optical density of said readable sample at 450 nm to determine whether said readable sample contains an elevated level of endogenous lactoferrin as compared to a reference value for healthy control subjects.

14. The diagnostic assay as recited in claim 13, wherein if said optical density of said readable sample is greater than or equal to 0.200, said fecal sample contains an elevated level of endogenous lactoferrin as compared to a reference value for healthy control subjects.

17. A kit for distinguishing irritable bowel syndrome from inflammatory bowel disease by testing a fecal sample from a person to be diagnosed, the kit comprising: one or more microassay plates, each said plate containing immobilized polyclonal antibodies to human lactoferrin; enzyme-linked polyclonal antibody to human lactoferrin; and enzyme substrate for color development.

20. A method for monitoring a patient having inflammatory bowel disease, the method comprising: obtaining a first fecal sample from the inflammatory bowel disease patient at a first time; determining the concentration of endogenous lactoferrin in said first fecal sample to obtain a first lactoferrin concentration; obtaining a second fecal sample from the inflammatory bowel disease patient at a second time later than said first time; determining the concentration of endogenous lactoferrin in said second sample to obtain a second lactoferrin concentration; and comparing said first lactoferrin concentration to said second lactoferrin concentration to evaluate any differences therebetween.